SANTA CRUZ BIOTECHNOLOGY, INC.

Intersectin siRNA (m): sc-41366

BACKGROUND

Intersectin, which is also designated Ese1 or ITSN1, is a component of the cellular endocytic machinery. Intersectin is composed of two N-terminal Ese15 homology (EH) domains, a central highly charged region and five C-terminal SH3 domains, which all largely contribute to the association of Intersectin with other components of the endocytic pathway. The EH domain is particularly responsible for the directed localization of Intersectin to Clathrin-coated pits near the plasma membrane. Within the endocytic vesicles the SH3 domains facilitate the binding of Intersectin with Dynamin, and the central domain is essential for the association of Intersectin with SNAP 25. Two isoforms of Intersectin are produced as a result of alternative splicing in a stop codon, and they are designated as Intersectin-short and long (or Intersectin$_S$ and Intersectin$_L$) to reflect an extended C-terminal domain. The long form, which has an extended C-terminal domain, is specifically expressed in neurons; the short form is detected in both glial and nonneuronal cells. The related proteins Intersectin-2 and the murine homolog Ese2 also contain the characteristic N-terminal EH domains, the central coiled-coil domain and five C-terminal SH3 domains and are likely involved the endocytic scaffolding complexes.

REFERENCES

1. Guipponi, M., et al. 1998. Two isoforms of a human Intersectin (ITSN) protein are produced by brain-specific alternative splicing in a stop codon. Genomics 53: 369-376.
2. Yamabhai, M., et al. 1998. Intersectin, a novel adaptor protein with two Eps15 homology and five Src homology 3 domains. J. Biol. Chem. 273: 31401-31407.
3. Hussain, N.K., et al. 1999. Splice variants of Intersectin are components of the endocytic machinery in neurons and nonneuronal cells. J. Biol. Chem. 274: 15671-15677.
4. Okamoto, M., et al. 1999. EHSH1/Intersectin, a protein that contains EH and SH3 domains and binds to Dynamin and SNAP 25. A protein connection between exocytosis and endocytosis? J. Biol. Chem. 274: 18446-18454.
5. Simpson, F., et al. 1999. SH3-domain-containing proteins function at distinct steps in Clathrin-coated vesicle formation. Nat. Cell Biol. 1: 119-124.
6. Sengar, A.S., et al. 1999. The EH and SH3 domain Ese proteins regulate endocytosis by linking to Dynamin and Eps15. EMBO J. 18: 1159-1171.

CHROMOSOMAL LOCATION

Genetic locus: Itsn1 (mouse) mapping to 16 C3.3.

PRODUCT

Intersectin siRNA (m) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Intersectin shRNA Plasmid (m): sc-41366-SH and Intersectin shRNA (m) Lentiviral Particles: sc-41366-V as alternate gene silencing products.

For independent verification of Intersectin (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-41366A, sc-41366B and sc-41366C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

Intersectin siRNA (m) is recommended for the inhibition of Intersectin expression in mouse cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 µM in 66 µl. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

Intersectin (29): sc-136242 is recommended as a control antibody for monitoring of Intersectin gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgGκ BP-FITC: sc-516140 or m-IgGκ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Intersectin gene expression knockdown using RT-PCR Primer: Intersectin (m)-PR: sc-41366-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.